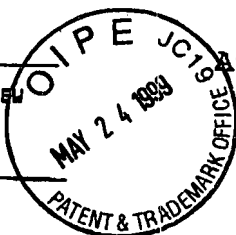


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 Assistant Commissioner for Patents,  
 Washington, D.C. 20231,  
 on

PATENT

TOWNSEND and TOWNSEND and CREW

By \_\_\_\_\_



Attorney Docket No. 16994-003123US

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of )

Herman A. DeBoer et al. )

Application No. 08/461,333 )

Filed June 5, 1995 )

For PRODUCTION OF RECOMBINANT  
 POLYPEPTIDES BY BOVINE SPECIES  
 AND TRANSGENIC METHODS )

Examiner: D. Crouch

Art Unit: 1819

DECLARATION OF  
FRANK PIEPER under \$1.132

Assistant Commissioner for Patents  
 Washington, D.C. 20231

Sir:

I, Frank Pieper, Ph.D., a co-inventor of the above-captioned application, state as follows.

1. My qualifications and experience in the design and application of tissue-specific expression vectors and transgenic animals are as stated in a previous declaration in the above-captioned case.

2. I understand the Examiner has requested evidence that promotor and enhancers of non-bovine origin are expected to be functional in expression and targeting of proteins to the mammary gland in bovine transgenic animals.

3. For purposes of expressing and targeting a protein encoded by a transgene to the mammary gland of an animal, it is, in my opinion, largely irrelevant whether the transgene contains

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promoter/enhancer sequences from the same or a different species as the animal. This opinion is based on the following facts.

(a) It is now clear that mammary-gland specific promoters are highly conserved between species including bovines. For example, Rosen et al., *Ann. New York. Acad. Sci.* 478, 63-76 (1986) at p. 70 reports that the 5' flanking region of three rat casein and the bovine  $\alpha$ -casein genes contains regions that are more highly conserved than the entire mature coding region of these genes. Transcription factors that bind to promoters are also highly conserved between species. The high degree of conservation of both promoters and transcription factors suggests that the transcription factors of one species can recognize the promoters of another. Thus, milk-specific promoters from one species are expected to be expressed in another.

(b) This expectation is entirely consistent with empirical evidence that has accumulated to date. There have been many reported examples in which promoter/enhancer sequences have been successfully used to target protein expression to the mammary gland of animals from a different species. These examples include numerous instances in which promoter/enhancer sequences from farm animals have been used to target proteins to the mammary gland in mice. Such experiments are routinely performed to test the efficacy of transgenes before introduction into larger animals. For example, Archibald et al., *Proc. Natl. Acad. Sci. USA* 87, 5178-5182 (1990) describe use of a sheep  $\beta$ -lactoglobulin promoter to express  $\alpha$ 1-antitrypsin in mice. Meade et al., *Bio/Technology* 8, 443-446 (1990) describe use of a bovine  $\alpha$ s1 casein promoter to express urokinase in mice. Stinnakre et al., *Fed. Eur. Biochem. Soc.* 284, 19-22 (1991) describe use of a bovine lactalbumin promoter to express trophoblastin in mice. Brem & Hartl, in *Frontiers of Biotechnology in Agriculture* (Israel, 1991) at p. 2 describe use of a bovine  $\alpha$ s1 casein promoter to express prochymosin in rabbits.

Numerous other examples show that promoter/enhancer sequences from rodents can be used to target proteins to the mammary gland of farm animals. For example, the whey acid promoter (WAP), which is found naturally in rodents but not in

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livestock species (pig, goat, sheep or cattle), has been used to express protein C and tPA in pigs and goats respectively (Drohan et al., *Thromb. Hemostasis* 65, 465 (1991); Ebert et al., *Bio/Technology* 9, 835-838 (1991). Further, an intact WAP gene has been expressed to give WAP in the milk of pigs (*Mech. Development* 36, 67-74 (1991)). Many other examples where promoter/enhancer sequences from one species have been used to target proteins to the milk in another species are provided in the Mammary Transgene Database at <http://www.biomedcomp.com>.

4. In summary, the above results establish a general rule that promoter/enhancer sequences from farm animals are functional in rodents and vice versa. This explains my conclusion that the species of origin of a promoter/enhancer is essentially irrelevant for obtaining expression of a protein in the milk of transgenic bovines.

5. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 19/12/97  
Frank Pieper, Ph.D.

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